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Subject: Public Comments on the Metal Carboxylates Coalition HPV Challenge Program Test Plan Revisions for the Metal Carboxylates Category.

The following comments on the Metal Carboxylates Coalition's revisions to its HPV Challenge Program test plan for the Metal Carboxylates Category are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal protection, and environmental organizations have a combined membership of more than ten million Americans.

We request that EPA reopen the comment period for the metal carboxylates test plans, since, as a result of breaking up the category, the numbers of animals to be used has greatly increased and there are a number of serious scientific and animal welfare concerns that need to be addressed.

We will be submitting additional comments on the new individual test plans but, in general, the animal protection community is quite concerned about these changes and the huge increase in the number of animals who will be killed to implement them. The EPA has just begun to post comments on the first of the new test plans in the last few months. The initial test plan proposed three mammalian toxicity tests, as well as two or three fish toxicity tests (the test plan seemed to contradict itself in one case). If carried out, these tests would have consumed approximately 750 mammals and 300 fish. However, the revised test plans submitted to date would cause far greater animal suffering. Four mammalian acute tests, three repeated dose tests, five combined repeated dose/reproductive/developmental tests and four in vivo genotoxicity tests are now proposed, as well as six fish acute tests. Together, these tests would consume approximately 4,000 mammals and 720 fish – a five-fold increase in the number of animals to be killed.

Many of these new tests appear to have been proposed only to confirm adequate existing data. In each test plan, a general discussion of the dissociation of metal carboxylates is presented. Experimental values for dissociation constants are given which conclusively show that each metal carboxylate is completely dissociated in the low pH environment of the mammalian stomach. It is concluded, as a result, that toxicity data for the free acid and metal ion, or their simple salts, can serve as surrogates for that of the respective metal carboxylate.

This would be especially applicable in the proposed tests which plan to use an oral exposure route. In the case of cobalt (II) salts in particular, the work of Stopford, et al.[1] is cited in the test plans. This study shows that cobalt chloride is similar to, or more bioavailable than, the corresponding cobalt carboxylate salts, thus making the chloride a conservative surrogate in estimating bioavailability and toxicity of the dissociated metal ion. Even though toxicity data for the dissociation products, or their simple salts, are summarized in many instances, thereby fulfilling the relevant endpoints, additional tests on the respective metal carboxylates are nevertheless inexplicably proposed.

Because the Metal Carboxylates Coalition submitted its original test plan in 2003, it may be unaware that a similar approach, using existing data on dissociation products, was subsequently endorsed by the EPA and all stakeholders in 2004 for E. I. du Pont de Nemours & Company's test plan for triisopropylborate, a compound which breaks down to isopropanol and boric acid in water (see http://www.epa.gov/oppt/chemrtk/triprobt/c14841tc.htm). This approach has been used in a number of other test plans as well in which compounds dissociate at low pH and the toxicity data on the dissociation products has been used to meet the SIDS requirements.

Four acute oral LD50 tests are proposed for various cobalt (II) carboxylates. In each case, acute mammalian toxicity data for the dissociation products or their simple salts are summarized in the test plan. In two cases, no justification whatsoever is offered for these new tests, while in the others, it is simply noted that the new data will support the "category approach" by confirming the existing data on the dissociation products. Clearly, this is not a "thoughtful, qualitative analysis" of existing data as required by the EPA in its October 1999 letter to chemical sponsors addressing animal welfare concerns and falls far short of justifying the exceptionally agonizing suffering that lethal dose testing inflicts on animals. Furthermore, the OECD guidelines which the proposed tests would follow are not specified. The coalition does not appear to be aware that OECD 401 has been phased out in favor of OECD 425 and that the EPA now recommends the use of in vitro cell toxicity tests to establish the starting dose for acute toxicity tests in order to further reduce the number of animals used.

The three, 7-day repeated dose tests and several of the combined repeated dose/reproductive/developmental tests are subject to similar criticisms. In its comments on the Aluminum Stearates Category test plan, the EPA specifically rejected the approach of conducting so-called "bridging" studies on the metal carboxylate in order to confirm existing data on its dissociation products, noting that "it is not clear how the proposed 7-day repeated-dose bridging study would demonstrate that the dissociation products data are representative of aluminum stearates toxicity". The comments also stress that the EPA "does not support further testing for mammalian toxicity endpoints." We are unaware of any OECD guidelines for a 7-day repeated dose test and none are specified in the test plans.

In addition to duplicating existing data for dissociation products, the four proposed in vivo genotoxicity studies ignore the EPA's guidance, set forth in its October 1999 letter and December 2000 *Federal Register* notice, that in vivo genotoxicity testing should be conducted only when known chemical properties preclude the use of in vitro testing and justification for doing so is provided. No justifications for the proposed in vivo tests are offered, and several test

plans reference a presumed in vivo genotoxicity endpoint, suggesting a disturbing lack of awareness of HPV program requirements.

In the case of the proposed fish acute toxicity tests, stearic and napthenic acids have partition coefficients greater than log KOW = 4.2, indicating that the tests may be unnecessary for the HPV program, as referenced in the December 2000 *Federal Register* notice. Further, no ecotoxicity data for aquatic plants or invertebrates exist for cobalt acetate or neodecanoic acid, cobalt salt. The fish test is intended to show whether exposure to these metal carboxylates will result in large-scale fish death thereby predicting economic loss and ecologic damage. If this exposure kills the food on which fish subsist, it could deplete fish populations even without direct fish toxicity. Since the toxicity of these metal carboxylates to aquatic plants and invertebrates is still unknown, tests on fish are premature. In addition, ECOSAR and non-animal ecotoxicity tests, such as the DarT test[2] and TETRATOX test[3] should be considered. In those cases for which fish acute toxicity tests are still perceived to be required, ECVAM's Ecotoxicology Task Force recently published an evaluation of a fish acute threshold (step-down) test concept with the potential to reduce the number of fish used in ecotoxicity testing by 53.6%-71.2%.[4]

In summary, the new test plans fail to justify the proposed animal tests, omit essential detail and demonstrate a disregard for HPV program policy. This suggests that the shortcomings evident in the initial Metal Carboxylates Category test plan have not been fully addressed and makes evident the urgent need for an adequate public comment period before the proposed testing is implemented and thousands of animals are killed.

Sincerely,

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[1] Stopford W., Turner J, Cappellini D, and Brock T. 2003. Bioaccessibility testing of cobalt compounds. J. Environ. Monit. 5(4): 675-680.
[2] Nagel, R. 2002. DarT: the embryo test with the zebrafish Danio rerio: A general model in ecotoxicology and toxicology. ALTEX 19 (Suppl. 1), 38-48.
[3] Schultz, T.W. 1997. TETRATOX Tetrahymena pyriformis population growth impairment endpoint: A surrogate for fish lethality. Toxicological Methods 7, 289-309.
[4] Jerama, S., et al. 2005. A strategy to reduce the use of fish in acute ecotoxicity testing of new chemical substances notified in the European Union. Regulatory Toxicology and Pharmacology 42, 218–224.